532 Rec'd PCT/PTO 10 OCT 2000

PCT

FORM PTO-1390 (REV 5-93) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
2296.2160	
U S APPLICATION NO (If known, see 37 C F R 1 5)	
09/673110	
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE
PCT/GB99/01080	8 April 1999 (08.04.99)
PRIORITY DATE CLAIMED	
9 April 1998 (09.04.98)	

TITLE OF INVENTION

ADHESIVES

APPLICANT(S) FOR DO/EO/US

Gordon Nelson and Christopher Andrew Jones

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the application time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(l).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:

U.S. APPLICATION NO. (If known, see 37 C F R 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/673110	PCT/GB99/01080	2296.2160

17. ☐ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5):		
Search Report has been prepared by the EP or JPO **$860.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.492(a)(1)) **$690.00**		
No international preliminary examination fee paid to USPTO (37 CFR 1.492 (a)(1)) but international search fee paid to USPTO (37 CFR 1.492(a)(2)) **$710.00**		
Neither international preliminary examination fee (37 CFR 1.492(a)(1)) nor international search fee (37 CFR 1.492(a)(2)) paid to USPTO **$1,000.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.492 (a)(4)) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$1,000.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	84 -20 =	64	X **$18.00**	$1,152.00	
Independent Claims	7 - 3 =	4	X **80.00**	$ 320.00	
Multiple dependent claim(s) (if applicable)			+ **$270.00**	$ 270.00	
			TOTAL OF ABOVE CALCULATIONS =	$	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
			SUBTOTAL =	$2,742.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
			TOTAL NATIONAL FEE =	$2,742.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
			TOTAL FEES ENCLOSED =	$	
				Amount to be:	
				refunded	$
				charged	$

a. ☒ A check in the amount of $2,742.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1205 . A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Fitzpatrick, Cella, Harper & Scinto
30 Rockefeller Plaza
New York, NY 10112-3801

SIGNATURE

NAME
Raymond R. Mandra

REGISTRATION NUMBER 34,382

2296.2160

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	:	Examiner: N/Y/A
Gordon NELSON et al	)	
	:	Group Art Unit: N/Y/A
Application No.: 35 USC 371 of PCT/GB99/01080	)	
	:	
Filed: April 8, 1999	)	
	:	
For: ADHESIVES	)	October 6, 2000

Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to examination on the merits, please amend the above-identified application as follows:

IN THE CLAIMS:

Please cancel claims 39-41.

Kindly amend claims 7-18 and 21-38 as follows:

7. (Amended) A composition [or method] according to claim 3 [or 6] in which the cofactor comprises a phenolic moiety which comprises at least one of a monohydroxy phenol group or a dihydroxy phenol group.

8. (Amended) A composition [or method] according to claim 3[,6, or 7] in which the cofactor is soluble in water.

9. (Amended) A composition [or method] according to claim 7[or 8] in which the cofactor comprises catechin.

10. (Amended) A composition [or method] according to [any of claims] claim 7 [to 9] in which the cofactor comprises catechol.

11. (Amended) A composition [or method] according to [any preceding claim] claims 1 or 2 in which the non-enzymatic bifunctional crosslinking agent comprises glutaraldehyde.

12. (Amended) A composition [or method] according to [any preceding claim] claims 1 or 2 in which the non-enzymatic bifunctional crosslinking agent comprises a di-isocyanate.

13. (Amended) A composition [or method] according to claim 12 in which the di-isocyanate is Trixene.

14. (Amended) A composition [or method] according to [any preceding claim] claims 1 or 2 in which the non-enzymatic bifunctional crosslinking agent comprises a quinone.

15. (Amended) A composition [or method] according to claim 14 in which the quinone is a benzoquinone.

16. (Amended) A composition [or method] according to [any preceding claim] claims 1 or 2 in which the phenol oxidase and the phenol hydroxylase is a tyrosinase.

17. (Amended) A composition [or method] according to claim 16 in which the tyrosinase is a mushroom tyrosinase.

18. (Amended) A composition [or method] according to claim 17 in which the mushroom tyrosinase is *Agaricus bisporus* tyrosinase.

21. (Amended) [Use of composition or] A method [according to any preceding claim] for binding substrates

together comprising adhering said substrates with a composition according to any one of claims 1, 2 or 19.

22. (Amended) [Use] A method according to claim 21 in which the substrates are non water-absorbent.

23. (Amended) [Use] A method according to claim 21 in which the substrates are water absorbent.

24. (Amended) [Use] A method according to claim 21 in which the substrates comprise a non water-absorbent substrate and a water absorbent substrate.

25. (Amended) [Use] A method according to claim [22 or 24] 21 in which [the non water-absorbent substrate or] at least one of said substrates comprise at least one of metal or plastic.

26. (Amended) [Use] A method according to claim [23 or 24] 21 in which [the water absorbent substrate or] at least one of the substrates comprise at least one of wood, leather, cotton, paper, carpet, or textile.

27. (Amended) [Use according to claim 21 as a binder of] A method for binding particulates comprising binding said particulates with a composition according to any one of claims 1, 2 or 19.

28. (Amended) [Use] A method according to claim 27 in which the particulates comprise at lease one of sand or glass fibre.

29. (Amended) [Use according to claim 21 as an [undercoat to] A method of undercoating a coating comprising applying the composition according to any one of claims 1, 2 or 19 to a substrate followed by application of said coating over said composition.

30. (Amended) [Use] A method according to claim 29 in which the coating is a paint.

31. (Amended) [Use] A method according to claim 29 in which the coating is an adhesive.

32. (Amended) [Use according to claim 21 as a suture] A method for closing a wound comprising applying the composition according to anyone of claims 1, 2 or 19 to said wound.

33. (Amended) [Use] A method according to claim 32 [in a method for closing a wound] wherein said composition applied to said wound forms a suture.

34. (Amended) [Use according to claim 21 as] A food product having a gelling agent comprising the composition according to anyone of claims 1, 2 or 19. [in food products].

35. (Amended) A pharmaceutical composition comprising a pharmaceutically active ingredient and a crosslinked adhesive composition according to any of claim 1, 2 or [to 3 or 7 to] 19.

36. (Amended) A kit for manufacture of an adhesive, the kit comprising separate components, wherein admixture of the separate components forms an adhesive composition according to any of claims 1, 2 or [to 3 or 7 to] 19.

37. (Amended) A kit for manufacture of an adhesive that comprises separate first and second components, the first component comprising an extensin protein, the second component comprising: either

a non-enzymatic bifunctional crosslinking agent; or a phenol oxidase and a phenol hydroxylase and optionally a cofactor;

wherein admixture of the first and second components forms a composition according to any of claims 1, or 2 [to 3 or 7 to 18].

38. (Amended) A kit for manufacture of an adhesive that comprises separate first and second components, the first component comprising an extensin protein, the second component comprising a non-enzymatic bifunctional crosslinking agent and a phenol oxidase and a phenol hydroxylase and optionally a cofactor, wherein admixture of the first and second components forms a composition according to [any claims 2, 3 or 7 to 18] claim 2.

REMARKS

The claims have been amended to remove improper multiple dependencies and correct other informalities. Favorable consideration is respectfully requested.

Applicants' undersigned attorney may be reached in our New York office at (212) 218-2100. All

correspondence should continue to be directed to our below-listed address.

Respectfully submitted,

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Registration No. 34,382

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Adhesives

The present invention relates to water resistant adhesives.

Adhesives are widely used both domestically and industrially. They are typically applied to dry first and second surfaces to bond the surfaces together, for example as bonding agents to bond together particulate matter, or to adhere other solid materials such as woods and metals. It is often desired to bond surfaces together when one or more of the surfaces is wet. Many adhesives are, however, less effective or ineffective if the surfaces that are to be bonded together are wet or if water is applied to the bond after it has been formed. Consequently, much effort has been spent trying to identify and develop adhesives which are effective in wet environments.

Marine mussels are able to attach themselves to a variety of surfaces under water forming strong and durable bonds with those surfaces. The precise mechanism by which mussels achieve this adhesion is not known. J.H.Waite has described the proposed involvement of catechol oxidase and a phenolic protein (Mussel Beards: a coming of age. Chemistry & Industry, 1991, 607-611). The mussel polyphenolic protein is a highly repetitive protein which comprises 80 tandem repeats of a decapeptide of the sequence: Ala-Lys-Pro-Ser-Tyr-Pro-Pro-Thr-Tyr-Lys. The mussel protein is particularly rich in the amino acid 3,4-dihydroxyphenyl-L-alanine (L-dopa). Catechol oxidase occurs extensively throughout nature and is known to catalyse the ortho hydroxylation of phenols and oxidation of the resulting catechols to *o*-quinones. Waite proposed, therefore, that catechol oxidase catalyses oxidation of the L-dopa residues in the mussel polyphenolic protein to L-quinone. It was suggested that adhesion results from a

combination of coupling of peptidyl-dopa to the bound surface and crosslinking of the polyphenolic protein by reaction of peptidyl-dopa-quinone with nucleophiles such as the e-amino group of lysine residues in the protein.

A catechol oxidase from mushrooms known as mushroom tyrosinase is available commercially and has been shown to hydroxylate tyrosine residues in synthetic decapeptides identical in sequence to the repeat sequences in the polyphenolic protein (Marumo K. and Waite J.H. (1986) Optimization of hydroxylation of tyrosine and tyrosine-containing peptides by mushroom tyrosinase. *Biochemica et Biophysica Acta* **872**, 98-103).

Large scale production of the mussel polyphenolic protein has been attempted with a view to production of a commercial adhesive which can be crosslinked by mushroom tyrosinase. Methods for isolation of the polyphenolic protein from mussels are described in US 4 496 397, US 4 585 585, and US 4 687 740. None of these methods, however, have proved to be commercially viable because the polyphenolic protein can only be isolated in small quantities.

WO 97/19141 describes a method of manufacture of an adhesive comprising crosslinking first and second molecules such as gelatin and chitosan, each having one or more aromatic groups, via at least one quinone group. Gelatin and chitosan are readily available and adhesives formed by this method show some resistance to water. However, the bonds formed are not as strong and durable as those formed by the mussel polyphenolic protein. A further disadvantage is that gelatin is not soluble in water at room temperature and has to be heated before use. Chitosan is only soluble at low pH.

It is desired therefore to produce bioadhesives which are effective in wet environments, which can be produced cheaply in bulk quantities, and which have improved properties over known readily produced bioadhesives.

Extensin proteins are hydroxyproline-rich glycoproteins present in the cell walls of dicotyledonous plants. The exact function of extensins is not known, but they have been proposed to play a role in the structure of plant cell walls. Extensins also accumulate in plant cell walls upon wounding and pathogenic attack and are therefore also thought to be involved in defence. It is known that extensins are inherently sticky and their adhesion to glass, polypropylene, and polycarbonate has been described in a paper by Miller, J.G. and Fry, S.C., (1993) (Spinach extensin exhibits characteristics of an adhesive polymer. *Acta Bot. Neerl.* **42**(2), 221-231). However, the mechanism of adhesion here appears to be a mixture of purely intramolecular forces such as hydrogen bonding and ionic interactions. Oxidative processes are not involved as the adhesion is not inhibited using reducing agents such as ascorbate, mercaptoethanol and dithiothreitol. Although native non-crosslinked proteins can adhere to inert substances, the set adhesive will be sensitive to moisture. Ultimately strength loss and bond breakage will become apparent. A successful commercial adhesive system must be crosslinked both to improve the strength of the adhesive and to ensure adhesion in wet environments and areas of high moisture.

Extensins have been proposed to form crosslinks by Fry, C.F., (1982) (Isodityrosine, a new crosslinking amino acid from plant cell wall glycoprotein. Biochem.J. **204**, 449-455). Fry suggested that hydrogen peroxide and a peroxidase enzyme such as horseradish peroxidase could be used to form isodityrosine bonds via an ether linkage between two tyrosine residues. Formation of dityrosine crosslinks via a biphenyl linkage between two tyrosines was not thought to occur. The formation of an isodityrosine bond and comparison of that linkage with the dityrosine linkage is represented in figure 1 of the accompanying drawings.

The proposed existence of such crosslinking suggests that extensins may form adhesives in the presence of

hydrogen peroxide and a peroxidase enzyme. However, it is most unlikely that formation of an adhesive based on addition of a cofactor such as hydrogen peroxide will be practical. Hydrogen peroxide is a relatively low viscosity liquid and would be very difficult to mix with other components of the adhesive which also tend to be relatively viscous. Hydrogen peroxide is also fairly reactive and it is likely that bond formation would occur too quickly after it is added to the other components of the adhesive. A further disadvantage of hydrogen peroxide is that it has a relatively short shelf-life. Any contamination from, for example, dirt would introduce bacteria, many of which contain catalase enzymes which breakdown hydrogen peroxide.

Peroxidase has also been proposed to be involved in the hydroxylation of L-tyrosine residues to L-Dopa (Klibanov A.M., Berman Z., and Alberti B.N. (1981) Preparative hydroxylation of aromatic compounds catalysed by peroxidase. *J.Am.Chem.Soc.* **103**, 6263-6264), suggesting an alternative role for peroxidase in crosslink formation similar to crosslink formation in the mussel polyphenolic protein adhesive. However, this reaction requires oxygen and dihydroxyfumaric acid and must be carried out at 0°C, otherwise non specific oxidation of other amino acids occurs. Use of peroxidase for crosslinking of extensin proteins has therefore not been thought to provide a viable way of producing a commercial adhesive.

It has surprisingly been found that extensin proteins have remarkable adhesive properties.

According to a first aspect of the invention there is provided a composition for use as an adhesive comprising:

an extensin protein; and either

a non-enzymatic bifunctional crosslinking agent; or

a phenol oxidase and a phenol hydroxylase.

According to a second aspect of the invention there is provided a composition for use as an adhesive comprising:

an extensin protein;

a phenol oxidase and a phenol hydroxylase; and

a non-enzymatic bifunctional crosslinking agent.

According to the invention there is also provided a method for forming an adhesive according to the first aspect of the invention which comprises admixing an extensin protein with either:

an amount of a non-enzymatic bifunctional crosslinking agent; or

an amount of a phenol oxidase and a phenol hydroxylase effective for inducing crosslinking of the protein.

Also according to the invention there is provided a method for forming an adhesive according to the second aspect of the invention which comprises admixing an extensin protein with an amount of a non-enzymatic bifunctional crosslinking agent, a phenol oxidase and a phenol hydroxylase effective for inducing crosslinking of the protein.

Each component of compositions according to the invention may be soluble in water.

When using a phenol oxidase and a phenol hydroxylase for crosslinking, it is possible to include as a cofactor a phenolic moiety which comprises at least one of a monohydroxy phenol group or a dihydroxy phenol group. Examples of phenolic moieties which comprise a dihydroxy phenol group include catechol and catechin. The cofactor should be soluble in water. The cofactor may be present at about 1% weight by volume of the composition.

The term "extensin protein" used herein is defined for the purposes of this application as covering:

(i) natural extensin proteins, such as plant extensins (for example carrot, spinach, etc.);

(ii) non-natural synthetic extensins, such as extensins produced chemically or by expression of recombinant DNA in a suitable host;

(iii) extensin derivatives (whether chemical or synthetic) which have amino acid sequences which differ from the extensin sequences by virtue of amino acid substitution, deletion, or addition, protease truncation or post

translational modification; but which retain extensin activity.

Natural extensin protein as referred to in (i) above is a plant protein rich in hydroxyproline, tyrosine and lysine residues. The content of hydroxyproline in carrot extensin is at least 50%, the content of tyrosine is at least 10.1%, and the content of lysine is at least 6.9%.

DNA encoding carrot extensin has been cloned (Chen J. and Varner J.E. (1985) An extracellular matrix protein in plants: characterisation of a genomic clone for carrot extensin. EMBO J. **4**, 2145-2151; Chen J. and Varner JE (1985) Isolation and characterisation of cDNA clones for carrot extensin and a proline-rich 33KDa protein. *Proc.Natl.Acad.Sci.USA* **82**, 4399-4403).

Derivatives of the extensin as referred to in (iii) above may be obtained by expression of a modified DNA including a modified extensin gene. A derivative of the extensin may be substantially free of carbohydrate.

The extensin protein may be present in the composition in an amount upto about 50% weight by volume of the composition, for example, the extensin protein may be present in the composition in an amount from about 20% to about 30% weight by volume of the composition.

A procedure for isolation of an extensin according to the invention from carrots is described below:

Carrot extensin proteins were isolated from cores (7mm) of phloem parenchyma sliced with a scalpel into 1mm thick slices. Carrot preparations (90g) (finely chopped or gently homogenised) were washed twice in distilled water and incubated in potassium phosphate buffer (5mM, pH 6.0) containing chloramphenicol (50mg/ml) for three days at 28°C with shaking. The extraction buffer was exchanged each day. On completion, the carrots were washed in distilled water and immersed in a solution (600ml) containing polyvinyl polypyrrolidone (PVPP) (9g) and dithiothreitol (DTT) (5mM, final concentration). The tissue was homogenised for two minutes in a blender, pelleted by centrifugation and washed

with eight litres of distilled water. The cell wall pellet was further extracted (3 times) with 200ml of a solution containing calcium chloride (0.2M final concentration), DTT (5mM final concentration) and PVPP (9g). On centrifugation the supernatants were pooled, filtered to remove any solid material and reduced to a volume of 50ml using ultrafiltration (10,000 molecular weight cut-off). The concentrated extract was dialysed overnight in distilled water at 4°C and further reduced in volume to 10ml by ultrafiltration. Each 10ml volume was adjusted with 1M tris/HCl (pH 8) to give a final tris concentration of 10mM. The material was then applied to a cation exchange column (12 x 1.5 cm) (CM-Sepharose CL-6B) previously equilibrated with tris/HCl (10mM, pH 8) and the protein fractions eluted using one column volume of tris/HCl (pH 8) followed by a linear gradient (60ml) of 10-300mM tris/HCl (pH 8). The elution profile was monitored at 280nm and the fractions (3ml) corresponding to each peak were pooled and dialysed overnight with sodium acetate (100mM, pH 6) at 4°C. The dialysed samples were freeze-dried and weighed before further analysis. In all purifications peak 1 from the cation exchange column was ignored as it was primarily made up of PVPP.

It is noted herein that peroxidases are not phenol oxidases. Peroxidases act by hydrolysing hydrogen peroxide. It is possible that oxygen liberated by this reaction can oxidise phenolics, but this oxidation does not occur by an enzymatic process. Phenol oxidation is not possible in the presence of a peroxidase without the addition of hydrogen peroxide. It is also noted herein that peroxidases are not phenol hydroxylases.

The phenol oxidase and the phenol hydroxylase may be a tyrosinase. The composition can contain at least 0.005% weight by volume of tyrosinase. Tyrosinase has the advantage that it is readily available from a variety of sources. The tyrosinase may be a mushroom tyrosinase. The mushroom tyrosinase may be *Agaricus bisporus* tyrosinase.

The non-enzymatic bifunctional crosslinking agent may be present in the composition from about 0.1% to about 5% weight by volume of the composition. The non-enzymatic bifunctional crosslinking agent may be any non-enzymatic bifunctional crosslinking agent capable of inducing crosslinking of a composition for use as an adhesive according to the invention. The non-enzymatic bifunctional crosslinking agent may be at least one of glutaraldehyde, a di-isocyanate, or a quinone. The di-isocyanate may be Trixene, for example Trixene BI 7986 (Baxenden). Trixene has the advantage that it is blocked and unreactive until the temperature reaches 130°C. Compositions according to the invention that comprise Trixene may therefore form a thermo-setting adhesive. The quinone may be a benzoquinone or a derivative thereof. The amount of the benzoquinone in the composition can be about 1% weight by volume of the composition. The benzoquinone may be 1,2-benzoquinone, 1,3-benzoquinone, or 1,4-benzoquinone.

It has been found that the rate of oxidation of groups may be conveniantly controlled by varying the amount of phenol oxidase and phenol hydroxylase that is added, allowing the rate of linkage to be readily controlled. The rate of linkage may also be controlled by varying the amount of the non-enzymatic bifunctional crosslinking agent.

When compositions according to the invention that contain the non-enzymatic bifunctional crosslinking agent form crosslinks, the non-enzymatic bifunctional crosslinking agent reacts with the ε-amino group of lysine residues in the extensin protein. Each molecule of non-enzymatic bifunctional crosslinking agent can react with upto two lysine residues. When the two lysine residues are in different protein molecules, crosslinks are formed between different protein molecules. When the two lysine residues are in the same protein molecule, crosslinks are formed within the same protein molecule.

When compositions according to the invention that comprise the phenol oxidase and the phenol hydroxylase form crosslinks, the phenol hydroxylase catalyses the

hydroxylation of the phenol group of tyrosine residues in the protein to form a dihydroxy phenol group and the phenol oxidase catalyses the oxidation of the dihydroxy phenol group to form a quinone group. The quinone group of the modified tyrosine residue may then react with the ε-amino group of two lysine residues in the protein. When one or both of the lysine residues are in the same protein molecule as the modified tyrosine residue containing the quinone group, intramolecular crosslinking occurs. When one or both of the lysine residues are in different protein molecules to the modified tyrosine residue containing the quinone group, intermolecular crosslinking occurs.

Tyrosinase has the advantage that it is a phenol hydroxylase and a phenol oxidase. It will be appreciated therefore that compositions according to the invention which comprise tyrosinase have the advantage that a separate phenol oxidase and a phenol hydroxylase do not have to be added to the composition because the phenol oxidase and the phenol hydroxylase activity are on a single molecule.

In the accompanying drawings:

Figure 1 shows the formation of an isodityrosine bond and comparison of that linkage with the dityrosine linkage;

Figure 2 shows the hydroxylation and oxidation of a tyrosine residue by tyrosinase and reaction of the oxidised tyrosine residue with the ε-amino group of a lysine residue in the same protein molecule.

When compositions according to the invention that comprise the phenol oxidase and the phenol hydroxylase further include a cofactor which comprises a phenolic moiety with a monohydroxy phenol group, the phenol hydroxylase catalyses the hydroxylation of the monohydroxy phenol group in the cofactor to form a dihydroxy phenol group and the phenol oxidase catalyses the oxidation of the dihydroxy phenol group to form a quinone group. The quinone group of the modified cofactor may then react with the ε-amino group of lysine residues in the protein to form crosslinks within the same protein molecule or between different protein molecules.

When compositions according to the invention that comprise the phenol oxidase and the phenol hydroxylase further include a cofactor which comprises a phenolic moiety with a dihydroxy phenol group, the phenol oxidase catalyses the oxidation of the dihydroxy phenol group of the cofactor to form a quinone group. The quinone group of the modified cofactor may then react with the ε-amino group of lysine residues in the protein to form crosslinks within the same protein molecule or between different protein molecules. Thus, a cofactor comprising a monohydroxy phenol group or a dihydroxy phenol group may be used to increase the number of crosslinks formed compared to compositions according to the invention which comprise the phenol oxidase and the phenol hydroxylase that do not include the cofactor. The number of crosslinks which can potentially be formed may also be increased by increasing the number of tyrosine and/or lysine residues in the extensin protein by recombinant means. However, care should be taken not to increase the number of crosslinks formed too much as over-crosslinked adhesives can be brittle.

It will be appreciated that compositions according to the invention that comprise a cofactor which includes a dihydroxy phenol group may form crosslinks in the absence of the phenol hydroxylase. Consequently, there is also provided according to the invention a composition for use as an adhesive which comprises:

an extensin protein;

a cofactor comprising a dihydroxy phenol group;

a phenol oxidase; and optionally

a non-enzymatic bifunctional crosslinking agent.

There is also provided a method for forming an adhesive which comprises admixing an extensin protein with an amount of a cofactor comprising a dihydroxy phenol group, a phenol oxidase, and optionally a non-enzymatic bifunctional crosslinking agent effective for inducing crosslinking of the protein.

According to the invention there is also provided a kit for manufacture of an adhesive, the kit comprising separate

components, wherein admixture of the separate components forms a composition according to the invention.

According to the invention there is also provided a kit for manufacture of an adhesive that comprises separate first and second components, the first component comprising an extensin protein, the second component comprising: either
a non-enzymatic bifunctional crosslinking agent; or
a phenol oxidase and a phenol hydroxylase and optionally a cofator;
wherein admixture of the first and second components forms a composition according to the invention.

Also according to the invention there is provided a kit for manufacture of an adhesive that comprises separate first and second components, the first component comprising an extensin protein, the second component comprising a non-enzymatic bifunctional crosslinking agent and a phenol oxidase and a phenol hydroxylase and optionally a cofactor, wherein admixture of the first and second components forms a composition according to the invention.

Adhesives formed using compositions according to the invention have excellent strength, durability and water resistant properties. They are readily produced in bulk quantities and at low cost. The strength of adhesives according to the invention has been found to be at least as good as the strength of conventional water resistant adhesives.

Adhesives according to the invention have been found to have several uses. Adhesives according to the invention may adhere to water-absorbent substrates and can be used to adhere such substrates together. Adhesives of the present invention may adhere to a substrate having a hydroxy aromatic, dihydroxy aromatic, hydroxy phenone or amino group and can be used to adhere such substrates together. For example the substrate may be wood, leather, cotton, paper, carpet, or a textile.

Adhesives according to the invention have also been found to adhere to non water-absorbent substrates and can be used to adhere non water-absorbent substrates to each other

or to water absorbent substrates. For example, the non water-absorbent substrate may be a metal or a plastic.

Adhesives according to the invention may be used to bind together particulates. Examples of particulates include sand and glass fibre.

Adhesives according to the invention may be used as an undercoat to a protective coating such as a paint or to a coating such as a non water-resistant adhesive. Undercoats comprising adhesives according to the invention have been found to have the advantage that the coating that is subsequently applied to the undercoat is significantly more resistant to the effects of water than coatings applied in the absence of the undercoat or to coatings applied to a conventional undercoat.

Adhesives according to the invention may be used as a suture to close wounds. Sutures formed according to the invention have the advantage that they are resistant to water and may have reduced antigenicity compared to conventional sutures.

Adhesives according to the invention may be used as a gelling agent in food products.

According to the invention there is also provided a pharmaceutical composition comprising a pharmaceutically active ingredient and a crosslinked adhesive composition according to the invention.

Figure Legends

Figure 1 shows the formation of an isodityrosine crosslink (in compound (2)) by hydrogen peroxide and peroxidase from two tyrosines (1). Formation of a dityrosine crosslink (in compound (3)) is not thought to occur.

Figure 2 shows a tyrosine residue (2) being hydroxylated (3) and subsequently oxidised (4) by tyrosinase, followed by reaction of the oxidised tyrosine residue (4) with the ε-amino group of a lysine residue (1) in the same protein molecule to form an intramolecular crosslink (5).

Claims

1. A composition for use as an adhesive comprising:
an extensin protein; and either
a non-enzymatic bifunctional crosslinking agent; or
a phenol oxidase and a phenol hydroxylase.

2. A composition for use as an adhesive comprising:
an extensin protein;
a non-enzymatic bifunctional crosslinking agent; and
a phenol oxidase and a phenol hydroxylase.

3. A composition according to claim 1 or 2 which further comprises a cofactor when the composition comprises a phenol oxidase and a phenol hydroxylase.

4. A method for forming an adhesive which comprises admixing an extensin protein with either:
an amount of a non-enzymatic bifunctional crosslinking agent; or
an amount of a phenol oxidase and a phenol hydroxylase effective for inducing crosslinking of the protein.

5. A method for forming an adhesive which comprises admixing an extensin protein with an amount of a non-enzymatic bifunctional crosslinking agent, a phenol oxidase and a phenol hydroxylase effective for inducing crosslinking of the protein.

6. A method for forming an adhesive which comprises admixing an extensin protein either with an amount of a cofactor, a phenol oxidase and a phenol hydroxylase effective for inducing crosslinking of the protein or with an amount of a cofactor, a non-enzymatic bifunctional crosslinking agent, a phenol oxidase and a phenol hydroxylase effective for inducing crosslinking of the protein.

7. A composition or method according to claim 3 or 6 in which the cofactor comprises a phenolic moiety which comprises at least one of a monohydroxy phenol group or a dihydroxy phenol group.

8. A composition or method according to claim 3, 6, or 7 in which the cofactor is soluble in water.

9. A composition or method according to claim 7 or 8 in which the cofactor comprises catechin.

10. A composition or method according to any of claims 7 to 9 in which the cofactor comprises catechol.

11. A composition or method according to any preceding claim in which the non-enzymatic bifunctional crosslinking agent comprises glutaraldehyde.

12. A composition or method according to any preceding claim in which the non-enzymatic bifunctional crosslinking agent comprises a di-isocyanate.

13. A composition or method according to claim 12 in which the di-isocyanate is Trixene.

14. A composition or method according to any preceding claim in which the non-enzymatic bifunctional crosslinking agent comprises a quinone.

15. A composition or method according to claim 14 in which the quinone is a benzoquinone.

16. A composition or method according to any preceding claim in which the phenol oxidase and the phenol hydroxylase is a tyrosinase.

17. A composition or method according to claim 16 in which the tyrosinase is a mushroom tyrosinase.

18. A composition or method according to claim 17 in which the mushroom tyrosinase is *Agaricus bisporus* tyrosinase.

19. A composition for use as an adhesive which comprises:
an extensin protein;
a cofactor comprising a dihydroxy phenol group;
a phenol oxidase; and optionally
a non-enzymatic bifunctional crosslinking agent.

20. A method for forming an adhesive which comprises admixing an extensin protein with an amount of a cofactor comprising a dihydroxy phenol group, a phenol oxidase, and optionally a non-enzymatic bifunctional crosslinking agent effective for inducing crosslinking of the protein.

21. Use of a composition or method according to any preceding claim for binding substrates together.

22. Use according to claim 21 in which the substrates are non water-absorbent.

23. Use according to claim 21 in which the substrates are water absorbent.

24. Use according to claim 21 in which the substrates comprise a non water-absorbent substrate and a water absorbent substrate.

25. Use according to claim 22 or 24 in which the non water-absorbent substrate or substrates comprise at least one of metal or plastic.

26. Use according to claim 23 or 24 in which the water absorbent substrate or substrates comprise at least one of wood, leather, cotton, paper, carpet, or textile.

27. Use according to claim 21 as a binder of particulates.

28. Use according to claim 27 in which the particulates comprise at least one of sand or glass fibre.

29. Use according to claim 21 as an undercoat to a coating.

30. Use according to claim 29 in which the coating is a paint.

31. Use according to claim 29 in which the coating is an adhesive.

32. Use according to claim 21 as a suture for closing a wound.

33. Use according to claim 32 in a method for closing a wound.

34. Use according to claim 21 as a gelling agent in food products.

35. A pharmaceutical composition comprising a pharmaceutically active ingredient and a crosslinked adhesive composition according to any of claims 1 to 3 or 7 to 19.

36. A kit for manufacture of an adhesive, the kit comprising separate components, wherein admixture of the separate components forms an adhesive composition according to any of claims 1 to 3 or 7 to 19.

37. A kit for manufacture of an adhesive that comprises separate first and second components, the first component

comprising an extensin protein, the second component comprising: either
a non-enzymatic bifunctional crosslinking agent; or
a phenol oxidase and a phenol hydroxylase and optionally a cofator;
wherein admixture of the first and second components forms a composition according to any of claims 1 to 3 or 7 to 18.

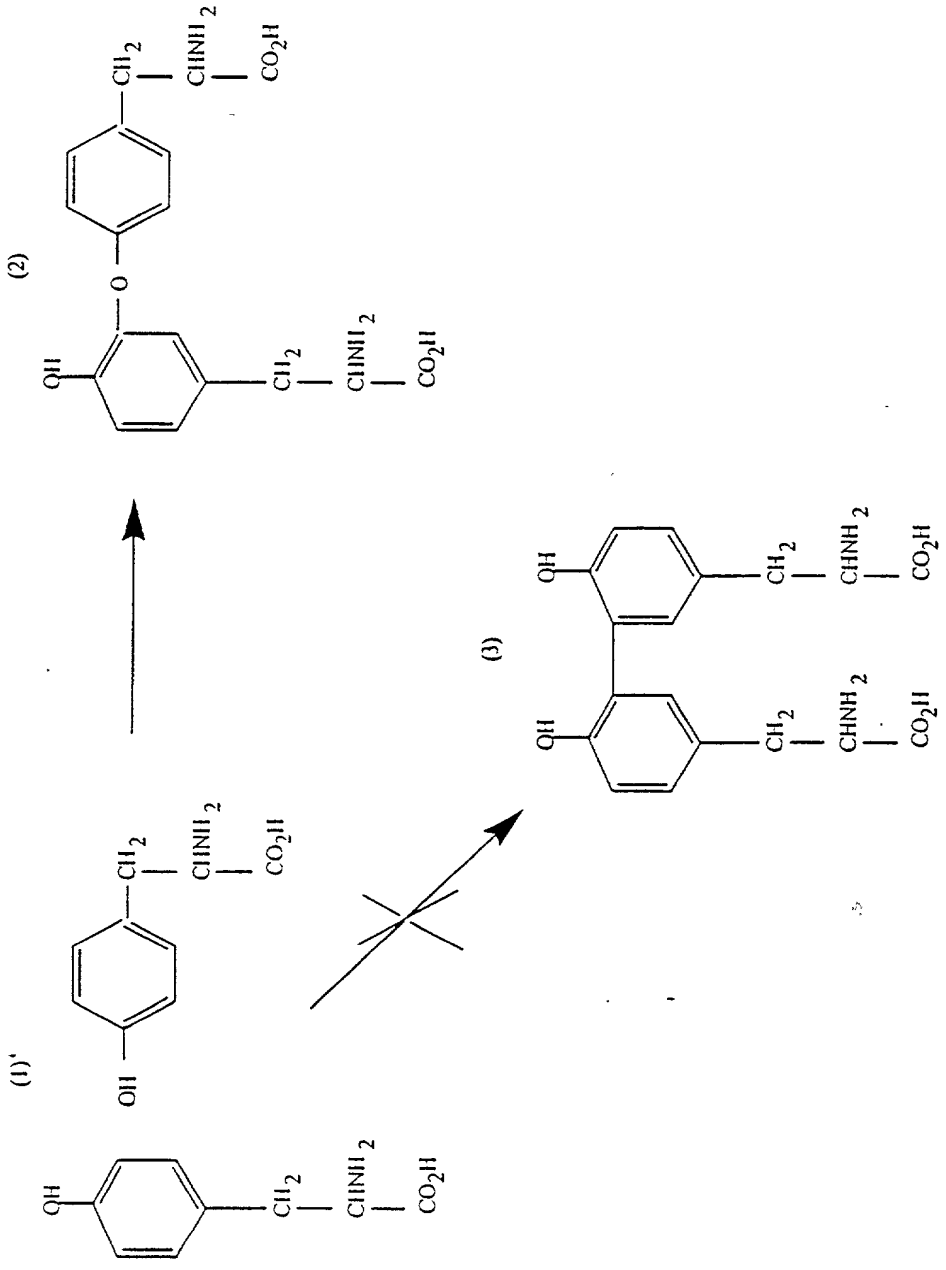
38. A kit for manufacture of an adhesive that comprises separate first and second components, the first component comprising an extensin protein, the second component comprising a non-enzymatic bifunctional crosslinking agent and a phenol oxidase and a phenol hydroxylase and optionally a cofactor, wherein admixture of the first and second components forms a composition according to any of claims 2, 3 or 7 to 18.

39. A composition for use as an adhesive substantially as described with reference to figure 2 of the accompanying drawings.

40. A method for forming an adhesive substantially as described with reference to figure 2 of the accompanying drawings.

41. A kit for manufacture of an adhesive substantially as described with reference to figure 2 of the accompanying drawings.

FIGURE 1



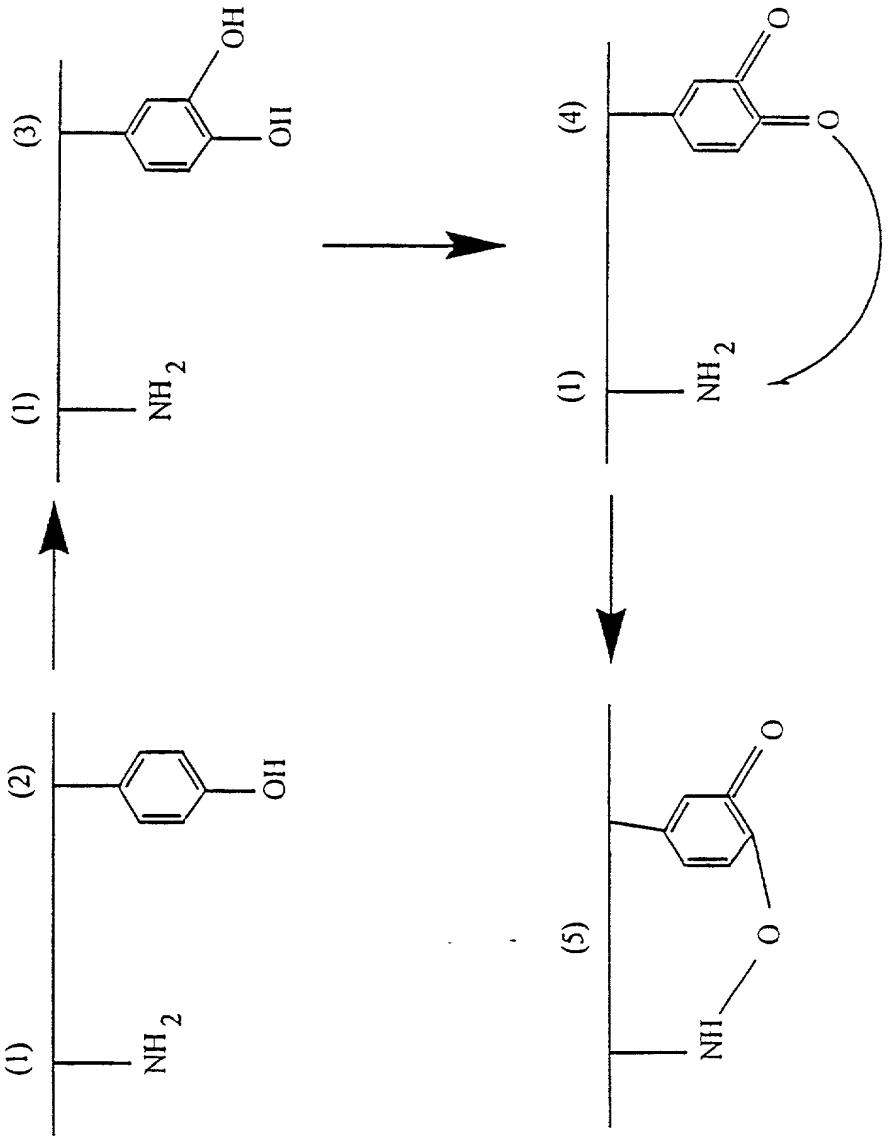


FIGURE 2

2296.2160

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT COOPERATION TREATY APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled ADHESIVES

the specification of which was filed as PCT International Application No. PCT/GB99/01080 on 8 April 1999 and was amended under PCT Article 19 on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Country	Application No.	Filed (Day/Mo./Yr.)	Priority Claimed (Yes/No)
U.K.	9807777.9	9 April 1998	YES

I hereby appoint the practitioners associated with the firm and Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to the address associated with that Customer Number:

FITZPATRICK, CELLA, HARPER & SCINTO
Customer Number: 05514

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Form #37

2296.2160

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT COOPERATION TREATY APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled ADHESIVES

the specification of which was filed as PCT International Application No. PCT/GB99/01080 on 8 April 1999 and was amended under PCT Article 19 on _____ (if applicable).

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Country	Application No.	Filed (Day/Mo./Yr.)	Priority Claimed (Yes/No)
U.K.	9807777.9	9 April 1998	YES

I hereby appoint the practitioners associated with the firm and Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to the address associated with that Customer Number:

FITZPATRICK, CELLA, HARPER & SCINTO
Customer Number: 05514

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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